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REVIEW

Looking with new eyes: advanced microscopy and artificial intelligence in reproductive medicine

Mark E. Gill[1] · **Alexander M. Quaas**[2]

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Abstract
Microscopy has long played a pivotal role in the field of assisted reproductive technology (ART). The advent of artificial intelligence (AI) has opened the door for new approaches to sperm and oocyte assessment and selection, with the potential for improved ART outcomes.

Keywords Advanced microscopy · Artificial intelligence (AI) · Assisted reproductive technology (ART) · Sperm selection · Oocyte assessment

Introduction

Since Antony van Leeuwenhoek first observed human sperm through an early compound microscope in 1677, microscopy has played an important role in the diagnosis and treatment of reproductive disorders. Today, the assessment of male factor infertility typically begins with a semen analysis (SA), assessing sperm number, motility, morphology, and vitality [1, 2]. All of these tests require microscopy for scoring, often via automated approaches (as in CASA, computer-assisted sperm analysis [3]) and sometimes via manual visual inspection. While each of the parameters examined via SA possesses some predictive power to diagnose male infertility, a substantial fraction of patients whose parameters fall within reference ranges for fertile men still struggle to have children. Recent approaches seek to improve the microscopy techniques performed on reproductive cells and the analysis of images obtained from such microscopy using artificial intelligence (AI)–based approaches.

✉ Mark E. Gill
mark.gill@fmi.ch

[1] Friedrich Miescher Institute for Biomedical Research (FMI), Maulbeerstrasse 66, 4058 Basel, Switzerland

[2] Division of Reproductive Medicine and Gynecological Endocrinology (RME), University Hospital, University of Basel, Basel, Switzerland

Conventional sperm morphology analysis

The percentage of sperm with "normal forms" is a standard parameter reported in SA. Normal sperm morphology has been defined by examining sperm bound to the cervical mucosa following coitus, with the assumption that sperm that can make its way rapidly to the upper portion of the female reproductive tract possesses a greater chance of fertilization. Analysis of sperm morphology requires extensive training and at least two sets of criteria: the "standard" WHO and the "strict" Kruger standards have been applied at different periods in the past. Even among men of known fertility, only a small fraction (4–14% in different versions of the WHO sperm analysis manual, with the lower bound of the most recent reference range being only 4% [1]) of sperm are often found to meet these strict morphological criteria.

The use of standard sperm morphological analysis as a predictive tool for fertility outcomes has shown varying results. A comprehensive literature review performed in 1998 showed a generally positive correlation between patients classified as falling within the WHO reference range and fertilization and pregnancy rates [4]. More recent studies suggest that the predictive power of sperm morphology may be limited, particularly in cases where intracytoplasmic sperm injection (ICSI) is utilized [5–7].

A major concern with the use of sperm morphology within conventional SA is the variability introduced in the assessment of sperm by different individuals [8]. A possible solution for this limitation is the introduction of

machine learning–based analysis approaches, in which a computer is trained by an expert to recognize sperm with optimal morphology. This approach could potentially provide any infertility clinic in the world access to the opinions of leading, experienced experts in sperm morphology analysis. Several algorithms have been trained using manually annotated sperm data sets, which contain information about common classes of sperm head morphologies as reported in the WHO manual [1, 9–11]. Each of these algorithms uses different approaches to identify and classify the morphologies present in the training set of images and obtain generally good accuracy in classifying sperm based on previous manual annotations. As with all machine learning–based approaches, the quality of the input data ultimately determines the quality and robustness of the outputs. Thus, as more data examining sperm morphology is generated and published, we can hope that the accuracy of these algorithms will improve further. Commercial, AI-driven semen analysis platforms already exist. However, a recent study examining one such system found substantial differences between manually determined values and those generated by the system [12]. Further, and larger, studies systematically comparing systems that can measure sperm morphology will be necessary to compare how these devices perform relative to the current gold standard of manual analysis.

Intracytoplasmic morphologically selected sperm injection (IMSI)

With the advent of ICSI, the choice of which individual sperm to use in embryo generation became an important question. Embryologists generally rapidly select motile sperm with normal shape when performing this technique. A modified version of ICSI, called IMSI (intracytoplasmic morphologically selected sperm injection), utilizes high powered (6000–10,000×) light microscopy to select sperm with more strictly defined "normal" morphology. Initial studies indicated that IMSI could improve success rates of assisted reproduction [13, 14], but a larger scale meta-analysis of several trials failed to find a significant improvement compared to conventional ICSI [15]. It has been suggested that IMSI may be useful in selected situations, for instance when sperm samples possess high levels of DNA fragmentation [16]. The argument against the use of IMSI as a standard procedure is the time and training required for its implementation. A highly trained embryologist must spend substantial time examining sperm prior to selection for each embryo to be generated. AI technologies may provide a solution to remove these limitations. Algorithms trained to recognize sperm as selected in IMSI (i.e., with few or no vacuoles [17]) could be used to identify sperm, which an embryologist could then pick for use in ICSI (following manual visual confirmation). These algorithms could potentially classify sperm faster than the human eye, allowing larger numbers of sperm to be screened prior to selection. The goal would thus be to integrate AI-based screening approaches in the microscopes used by embryologists for micro-injection, allowing for sperm selection recommendations validated by the embryologist and resulting in the use of sperm with the highest developmental competence.

Investigating sperm motility with AI

Sperm motility is another major feature measured in conventional SA. While this parameter was originally measured manually in all clinics, CASA for measuring sperm motility has become standard in many clinics around the world [3]. Video analysis using AI offers the possibility to examine differences more accurately (and rapidly) in the way that sperm move.

A recent study used AI to measure sperm motility in a set of videos taken from 85 men in Norway [18]. This study found that the best performing algorithm was able to match or exceed the performance of standard approaches in the classification of motility. Of note, the full analysis (including sample processing) required only 5 min, substantially faster and with less effort than that required for a conventional motility analysis.

Another recent study compared classification of sperm motility performed by a cloud-based AI system to grading generated by a well-trained expert in 47 individuals [19]. A correlation of 0.90 was found between expert-generated grades and automatically calculated sperm motility percentage, suggesting this approach may provide a rapid and less expensive alternative approach to conventional SA. The system tested in this study was designed for at-home SA, using smartphone cameras to capture the movies used for their analysis, an interesting possible extension to clinical SA. More frequent analyses may be possible if testing is performed by the patient directly, giving a more accurate picture of semen quality over time.

AI may allow identification of subtle differences in sperm motility patterns, allowing classification into multiple groups and perhaps revealing the underlying etiology of motility defects. The data set used in Hicks et al. included additional demographic and hormonal parameters [18], which did not improve classification of motility when added to the data set used to train their algorithm. This rich data set has been made available to the public [20],

allowing other researchers to try other approaches [21], with the potential to identify differences in sperm motility patterns associated with features such as BMI or sex hormone levels.

Machine learning algorithms to see beyond the obvious

Perhaps the most exciting possibility for using AI in sperm selection lies in its exquisite ability to detect minute differences in images. This means that it is in theory possible for an algorithm to observe differences in sperm, not obvious to a human observer. Several sperm features have been shown to negatively influence reproductive outcomes. Many of these features are, however, only detectable following staining and fluorescence microscopy. Sperm processed for such assays are generally not suitable for use in ART, as processing may involve fixation or denaturation toxic to sperm, and even in cases where live-imaging is performed, the presence of fluorescent dyes in sperm could impair embryonic development.

By performing assays for sperm function using fluorescence microscopy, while simultaneously acquiring bright-field microscopic images of sperm morphology, it is possible to identify subtle features in these bright-field images that can predict the presence features with a negative impact on embryonic development and health. A study using this rationale, examining sperm DNA fragmentation, associated with increased rates of recurrent pregnancy loss [22], was recently performed [23]. McCallum et al. used acridine orange (AO) staining (a method which localizes damaged DNA following denaturation by heat or acid [24, 25]) to identify sperm with high DNA fragmentation, and then trained an algorithm to predict the AO level based solely on a bright-field image [23]. This algorithm demonstrated a moderate ability to identify sperm with differing levels of DNA fragmentation. Conversely, DNA fragmentation levels were not predictable just from normal morphology (as judged by a trained expert), suggesting that this approach adds additional information relative to standard morphological examination.

While exciting, adoption of these approaches in the clinic likely requires more work. The AO staining protocol involves denaturation with acid and detergent, which could alter the shape of sperm, meaning that a fully untreated sample may not possess the same variation identified here. Secondly, to be useful in clinical application features identified by the algorithm would need to be robustly identified in living, motile sperm, which may prove difficult. However, further refinement of such methods should be possible and may provide clinical embryologists a very useful aid in improving overall ICSI success rates.

Evaluation of oocyte developmental competence

As standards in ART have moved toward single embryo transfer [26], efforts to improve non-invasive embryo selection represent a pivotal area of research in the field of reproductive medicine. Studies in animal models have shown that oocytes with varying DNA configurations differ in their capacity to support the development of an embryo [27, 28]. These configurations are not readily visible in unperturbed light microscopy images. Recent work using mouse oocytes has shown that training AI algorithms with fluorescence microscopy images allows for highly accurate identification of oocytes with a higher developmental potential [29]. A study training an algorithm using time-lapse images of unstained mouse oocytes found many features that could sub-divide mouse oocytes of differing qualities, including zona pellucida texture and area of the perivitelline space [30]. This algorithm was then applied to examine human oocytes that failed to properly respond to hormone stimulation [30]. They found that the texture of the zona pellucida was also associated with a lower chance to complete meiotic maturation. A major caveat to this analysis is that the examined oocytes were excluded from use in ART due to lack of maturation; however, these results suggest that features visible in light microscopy of the oocyte could be predictive of oocyte quality. Future investigations will seek to further correlate imaging of oocytes prior to ART and subsequent reproductive performance with regard to fertilization and embryo development. One challenge for training algorithms using oocyte information, as opposed to sperm, is the fact that numbers will be substantially more limited, and thus, imaging training sets will be of substantially smaller size, making robustness more challenging to establish.

Looking to the future: alternative imaging approaches

The approaches explained above generally focus on using standard light microscopy with more advanced analysis approaches to improve understanding of these images. A myriad of alternative imaging approaches already exists, with diverse clinical and research applications in humans and other species (Table 1, adapted and modified from [31]). These approaches hold the potential to reveal novel and dynamic changes in reproductive tissues, such as oocytes and embryos, with promise for basic reproductive research and clinical ART. As an example, fluorescence lifetime imaging microscopy (FLIM) has been used

Table 1 Non-invasive imaging approaches of gametes and embryos for use in reproductive research and ART (adapted and modified from [31])

Imaging approach	Information obtained	Species
Polarized light microscopy	-Oocyte spindle formation/location -Oocyte and embryo zona layers -Sperm acrosomal status -Sperm head vacuoles - sperm DNA arrangement	Mouse Hamster Rat Bovine Human Insect
Fluorescence lifetime imaging microscopy (FLIM)	-Detection of metabolic differences in blastocysts	Mouse Human
Multi-photon excitation (MPE)	-Oocyte and embryo mitochondrial distribution -Embryo cell lineage	Rhesus Mouse
Second-harmonic imaging microscopy (SHIM)	-Spindle dynamics -Oocyte zona layers -Oocyte organelle localization -Embryo lipid droplet distribution	Mouse
Fourier transformed infrared spectroscopy (FTIR)	-Oocyte zona pellucida secondary protein structure -Oocyte lipid phase transition temperature	Human Bovine Porcine Murine
Raman spectroscopy	-Sperm mitochondrial status -Sperm DNA damage -Oocyte oxidative damage -Oocyte quality -Oocyte maturational status	Fish Human Mouse Xenopus Sheep
Coherent anti-stokes Raman spectroscopy (CARS)	-Oocyte lipid content	Mouse Bovine Porcine Human
Optical quadrature microscopy (OQM)	-Embryo cell counts	Mouse
Phase subtraction: combination of differential interference controls (DIC) and optical quadratic microscopy (OQM)	-Embryo cell boundaries/overlaps	Mouse
Optical coherence tomography (OCT)	-Clumping of unknown cytoplasmic structures following embryo vitrification	Mouse
Biodynamic imaging (BDI)	-Cumulus cells, oocyte, zygote, and blastocyst observation of subcellular motion	Porcine
Quantitative orientation independent microscopy: combination of differential interference controls (DIC) and polarization microscopy	-Spermatocyte microtubule and chromosome distribution	Crane Fly
Multimodal microscopy: three-dimensional combination of multiple imaging modalities on one common platform, for example, DIC, epifluorescence, OQM, laser scanning confocal, two-photon	-Imaging of blastocysts	Mouse

to study oocyte mitochondrial dysfunction in a knockout mouse model [32], and to identify differences in metabolic signatures between euploid and aneuploid human blastocysts [33]. Advances in this area are likely to pave the way for new insights into cell cycle dynamics and novel tools for the non-invasive assessment of sperm, oocytes, and embryos.

Conclusion

Advanced microscopy in the field of ART is rapidly evolving with the advent of AI, with great promise for new approaches to sperm, oocyte, and embryo assessment and selection, and the potential for improved ART outcomes.

Data Availability Data sharing not applicable as no novel datasets were generated or analysed for this article.

Declarations

Conflict of interest Ferring Pharmaceuticals—Consultant/Speaker (AMQ).

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